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# Morphological Characters of the Trematode Larvae Similar to *Paragonimus* Found in the Snail, *Bythinella* (*Moria*) *nipponica* Mori, 1937 as the Intermediate Host of *Paragonimus miyazakii* Kamo *et al.*, 1961

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*Paragonimus miyazakii* was described by Kamo *et al.* (1961) as the fourth species of the genus *Paragonimus* in Japan. They obtained the adult worms from a cat and an albino rat which were experimentally fed with the new metacercariae, which were found from the fresh water crabs (*Potamon dehaani*) collected at Rokuroshi, Iwakuni City in Yamaguchi Prefecture. The crab, *Potamon dehaani* has so far been known as the only species of the second intermediate host of this fluke.

The natural first intermediate host had never been known for a long period of time, while *Oncomelania nosophora* has shown to be experimentally infected with the miracidia of this fluke (Kawashima et Miyazaki, 1964; Hatsushika *et al.*, 1966 a, b). In the meantime Hatsushika *et al.* (1966 b) reported a minute fresh water snail, *Bythinella* (*Moria*) *nipponica akiyoshiensis* (Kuroda et Habe, 1957) as the first intermediate host of *P. miyazakii*.

In the snails collected at the type locality the authors had found two types of larvae, of which one was identified as *P. miyazakii* and other *Paragonimus*-like larvae was not identified.

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In addition, the snails harboring the unidentified larvae were also found in other places such as Kamino, Mine City in Yamaguchi Prefecture, and Nishimori, Uma County in Ehime Prefecture.

Detailed morphological informations about these larvae are given in this paper for an intermediate report.

## Materials and Methods

The snails, *Bythinella* (*Moria*) *nipponica* Mori, 1937 were collected at Rokuroshi, Iwakuni City, the type locality of *P. miyazakii* Kamino, Mine City in Yamaguchi Prefecture, and Nishimori, Uma County in Ehime Prefecture from October, 1967 to September, 1969.

To obtain the larvae of *Paragonimus* parasitic in the snail, the shell was removed from the snail tissues, and then the larvae were isolated from the tissues including the liver and other glands in Petri dish with normal saline. The larvae removed from the snail tissues were studied under a microscope without stain or in vital stain with neutral red (1%) or toluidine blue (0.5%, pH 2.0). All the specimens from the snail were measured under the pressure of a microcover slip.

## Results

The infection rate of larva of *P. miyazakii* and unidentified one in *Bythinella* snails, collected at the type locality of *P. miyazakii* were shown in Table 1. Of 1958 snails examined, 67 or 3.4% were infected with the larvae of *P. miyazakii* and 28 or 1.4% were infected with unidentified larvae. The infection rate of *P. miyazakii* ranged from 1.8% to 7.5% with an average of 3.4%, and that of unidentified larvae ranged from 0.3% to 3.2% with an average of 1.4%. In addition, one of 1,409 snails (0.07%) collected from Kamino and two of 200 snails (1.0%) from Nishimori were infected with the unidentified larvae. However, there were no snails infected simultaneously with the both types of larvae. No sporocyst was found in the snails examined during the period of this investigation.

Measurements of the first and the second generation rediae (4 living specimens, each) were shown in Tables 2 and 3. The unidentified present rediae were elongated ellipsoids with a somewhat rounded posterior end, and they were morphologically very similar to those of *P. miyazakii*, and no distinct difference was found between them in external appearance expect that it is slightly small.

The first generation redia was somewhat smaller than that of *P. miyazakii*, though the external features of the rediae closely resemble those of the genus *Paragonimus*. The distinct difference between two types of rediae is recognized in the length of the intestine. As shown in Table 2, the intestine of *P. miyazakii* rediae is almost twice as long as the present rediae.

In the second generation rediae, the morphological difference between them is recognized clearly in the size of pharynx. The pharynx of the second generation rediae

Table 1 Incidence of *P. miyazakii* and unidentified present larvae in *Bythinella* snails collected at the type locality of *P. miyazakii*

| Year | Month | No. of snail examined | No. of snail infected (%) | |
|---|---|---|---|---|
| | | | *P. miyazakii* | Present larvae |
| 1967. | Oct. | 821 | 15(1.8) | 5(0.6) |
| 1968. | May | 324 | 14(4.3) | 10(3.1) |
| 〃 | July | 284 | 11(3.9) | 9(3.2) |
| 〃 | August | 40 | 3(7.5) | 1(2.5) |
| 1969. | July | 136 | 6(4.4) | 2(1.5) |
| 〃 | Sept. | 353 | 18(5.6) | 1(0.3) |
| Total | | 1,958 | 67(3.4) | 28(1.4) |

Table 2 Measurements (in microns) of lst generation rediae

| | *P. miyazakii* | Present larvae |
|---|---|---|
| | range (average) | range (average) |
| Body length | 310-350(330.0) | 189-400(283.7) |
| width | 80-125(102.5) | 80-100( 89.6) |
| Pharynx length | 57- 59( 58.0) | 49- 54( 51.9) |
| width | 57- 59( 58.0) | 51- 59( 54.0) |
| Intestine length | 120-149(134.5) | 54-113( 74.7) |
| No. of redial embryo | 6 ( 6 ) | 5- 7( 6 ) |

Table 3 Measurements (in microns) of 2nd generation rediae

| | *P. miyazakii* | Present larvae |
|---|---|---|
| | range (average) | range (average) |
| Body length | 340–610(460.1) | 480–610(557.5) |
| width | 70–150(123.7) | 110–130(122.5) |
| Pharynx length | 49– 70( 60.2) | 35– 46( 39.2) |
| width | 49– 65( 57.1) | 38– 46( 42.2) |
| Intestine length | 49–200( 98.2) | 81–122(105.8) |
| No. of cercarial embryo | 1– 8( 5 ) | 2– 5( 4 ) |

Table 4 Measurements (in microns) of cercariae

| | *P. miyazakii* | Present larvae |
|---|---|---|
| | range (average) | range (average) |
| Body length | 192–229(213.8) | 129–207(164.8) |
| width | 62–103( 86.2) | 45– 98( 71.1) |
| Oral sucker length | 38– 62( 46.6) | 35– 42( 37.3) |
| width | 35– 62( 47.1) | 32– 60( 39.9) |
| Ventral sucker length | 22– 49( 32.2) | 22– 36( 31.8) |
| width | 22– 33( 28.4) | 30– 37( 33.5) |
| Stylet length | 22– 35( 29.5) | 22– 28( 25.7) |
| width | 5– 7( 5.5) | 5– 7( 5.3) |
| Tail length | 15– 22( 17.6) | 9– 15( 11.6) |
| width | 11– 16( 13.2) | 9– 13( 10.9) |

of *P. miyazakii* ranged from 49 μm to 70 μm in length with an average of 60 μm, and 49 μm to 65 μm in width with an average of 57 μm. The pharynx of the present rediae, on the other hand, is smaller than that of *P. miyazakii* (Table 3). Moreover, the length of the pharynx of *P. miyazakii* has a tendency to be longer than the width, while that of the present rediae is wider than the length.

The unidentified cercaria is microcercous type with a very short and pyriform tail, and the entire body is covered with small spines. Measurents of the fully developed cercariae (15 living specimens, each) were shown in Table 4. In the present cercariae, the oral sucker measures 35 μm to 42 μm in length with an average of 37 μm, and 32 μm to 60 μm in width with an average of 40 μm. The stylet is clearly visible in the dorsal part of the oral sucker, and it averages 26 μm in length and 5 μm in width. The acetabulum is slightly smaller than the oral sucker. The central groove of the hind body is not so clear as that of *P. miyazakii*. In the external form, the present cercaria and the cercaria of *P. miyazakii* resemble each other expect that the former is definitely smaller than the latter. However, the present cercariae differ from those of *P. miyazakii* in the shape of excretory vesicle and the arrangement of penetration gland cells.

The excretory vesicle of the present cercaria is Y-shaped, and it may appear as a thick-walled sac, opening at the posterior end of the body. Two rows of penetration gland cells situated at the lower level of the ventral sucker on both sides of the body (Figs. 1, 7 and 8). It is assumed that the

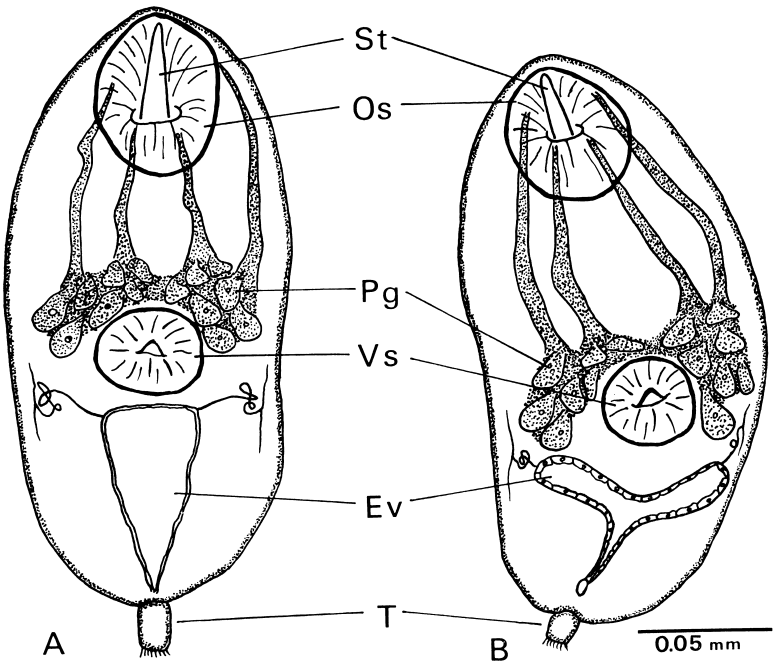


Fig. 1 Mature cercariae of *P. miyazakii* (A) and unidentified one (B) showing the shape of excretory vesicle, ventral view.
Ev : excretory vesicle Os : oral sucker Pg : penetration gland cells St : stylet T : tail Vs : ventral sucker

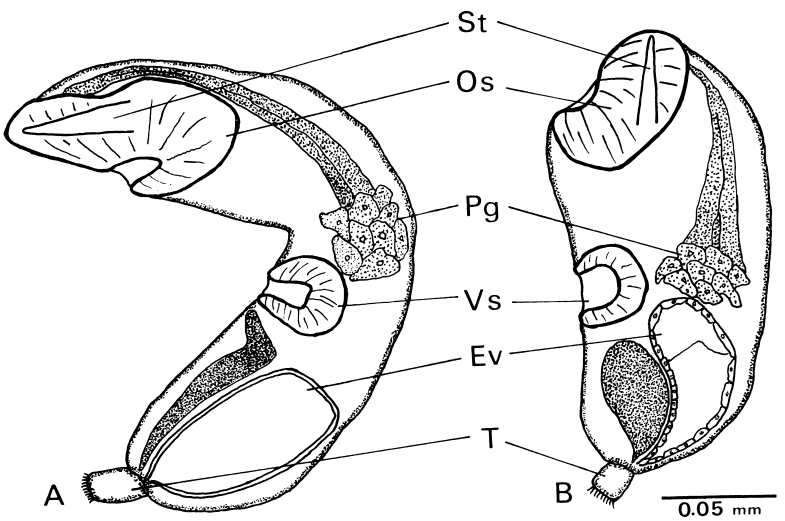


Fig. 2 Mature cercariae of *P. miyazakii* (A) and unidentified one (B) stained with 1% neutral red, said view.
Ev : excretory vesicle Os : oral sncker Pg : penetration gland cells St : stylet T : tail Vs : ventral sucker

Table 5 Measurements (in microns) of 2nd generation rediae of *P. miyazakii* reported by various authors

| Snail host | Body | | Pharynx | | Intestine length | No. of cercarial embryo | Authors |
|---|---|---|---|---|---|---|---|
| | length | width | length | width | | | |
| *Oncomelania nosophora* | 526– 650 (582) | 78–104 (94) | 46–65 (56) | 42–52 (47) | 111–182 (141) | | *Kawashima et Miyazaki (1964) |
| *O. nosophora* | 500–1,030 (769) | 90–225 (146) | 60–83 (72) | 50–70 (61) | 120–162 (147) | 4–8 (5) | *Hatsushika *et al.* (1966 b) |
| *Bythinella* | 490– 700 (580) | 112–140 (125) | 51–68 (65) | 50–59 (57) | 85–163 (131) | 4–7 (6) | Hatsushika *et al.* (1966 b) |
| *Bythinella* | 475 | 147 | 35 | 37 | 75 | | Hashiguchi (1967) |
| *Bythinella* | 514 | 137 | 33 | 35 | 49 | | |
| *Bythinella* | 509 | 132 | 35 | 54 | | | |
| *Bythinella* | 165– 305 | 50– 90 | 40–50 | 39–48 | | | *Hashiguchi et Miyazaki (1968) |
| *Bythinella* | 327 | 95 | 33 | 32 | 50 | 5 | Habe et Hamajima (1971) |

* Experimental infection

Table 6 Measurements (in microns) of cercariae of *P. miyazakii* reported by various authors

| Snail host | Body | | Oral sucker | | Ventral sucker | | Stylet length | Tail length | Authors |
|---|---|---|---|---|---|---|---|---|---|
| | length | width | length | width | length | width | | | |
| *Oncomelania nosophora* | 131–181 (161) | 70–82 (74) | 33–38 (35) | 35–40 (38) | 28–34 (32) | 30–35 (33) | 27–32 (30) | 16–20 (18) | *Kawashima et Miyazaki (1964) |
| *O. nosophora* | 198–243 (220) | 60–83 (72) | 45–55 (50) | 48–55 (53) | 35–43 (39) | 38–45 (41) | 26–30 (28) | 13–18 (17) | *Hatsushika *et al.* (1966 b) |
| *Bythinella* | 151–259 (202) | 68–97 (76) | 43–62 (47) | 35–48 (41) | 33–40 (37) | 30–37 (35) | 24–30 (27) | 14–18 (16) | Hatsushika *et al.* (1966 b) |
| *Bythinella* | 230 | 84 | 48 | 49 | | | | | Hashiguchi (1967) |
| *Bythinella* | 171 | 65 | 38 | 44 | 31 | 34 | 26 | 15 | |
| *Bythinella* | 197 | 73 | 39 | 42 | 30 | 31 | 24 | 17 | |
| *Bythinella* | 163–253 | 53–95 | 39–53 | 33–58 | 23–40 | 25–38 | | 18–25 | *Hashiguchi et Miyazaki (1968) |
| *Bythinella* | 177–249 (218) | 68–87 (77) | 35–58 (46) | 38–61 (49) | 30–45 (38) | 32–46 (39) | 27–30 (28) | 14–20 (17) | Nishida *et al.* (1969) |
| *Bythinella* | 128–164 (142) | 61–84 (69) | 32–40 (36) | 21–38 (33) | 18–25 (22) | 72–30 (29) | 19–24 (21) | 5–13 (8) | Habe et Hamajima (1971) |

* Experimental infection

penetration gland cells is almost the same as that observed for *P. miyazakii*, but further study on this point is necessary. The flame cell formula of the present cercaria is still unknown. Generally speaking, it is rather difficult to distinguish the present cercariae from those of *P. miyazakii* by external features. However, it is distinguishable from that of *P. miyazakii*, when Y-shaped excretory vesicle was found in the fresh or stained specimen.

Reactions of *P. miyazakii* and the present cercariae for toluidine blue (0.5%) were also examined. As the metachromatic reactions, the mucoid substances of the present cercariae gave an appearance of the thick basal

layer in the hind body (Fig. 10), while those of *P. miyazakii* took the appearance of the very thin layer in the ventral surface of the body (Fig. 6).

## Discussion

The structure of the first and the second generation rediae and the cercariae of *P. miyazakii* found in *Bythinella* snails are identical with those reported by Kawashima et Miyazaki (1964) and Hatsushika *et al.* (1966 a, b) from the experimentally infected snails, *O. nosophora* (Tables 5 and 6). The present larvae found in *Bythinella* snails were significantly distinguishable from those of *P. miyazakii* in the length of intestine (the first generation redia), in the size of pharynx (the second generation redia), in the shape of excretory vesicle, in the arrangement of penetration gland cells (the cercaria).

Hatsushika *et al.* (1966 a, b) and Kamo *et al.* (1967 a) suggested that the length of the pharynx of the second generation redia of *P. miyazakii* was longer than the width, unlike in most cases of other species of *Paragonimus*. The same applies to the second generation rediae of *P. miyazakii* in the present report (Table 3).

Kamo *et al.* (1967 b) reported on the distribution of mucoid gland cells and mucoid reservoirs in the cercariae of *P. miyazakii*. Comparative studies of mucoid gland cells between *P. miyazakii* and the present cercariae will be reported separately in the future.

The second generation rediae and the cercariae of *P. miyazakii* were described by several workers (Tables 5 and 6). Although we cannot draw definite conclusion on this point, some cases in these tables indicate a possibility of the confusion of *P. miyazakii* with the present larvae. For instance, Hashiguchi (1967) reported some inconsistent results of the studies on the infection rate of the cercaria and the metacercaria of *Paragonimus* larvae in one locality of Fukuoka Prefecture, in which the infection rate of the cercaria in *Bythinella* snails was considerably higher than that of the metacercaria in crabs. Miyahara et Kawashima (1974) also reported two types of cercariae with different length of the stylet found from *Bythinella* snails in the suburbs of Fukuoka City.

On the other hand, the present cercariae are somewhat similar to those of the family Nanophyetidae (Wallace, 1935) Dollfus, 1939, which were reported by Witenberg (1932), Wallace (1935), Bennington et Pratt (1960), and Millemann et Knapp (1970). Recently, Saito *et al.* (1977) reported the occurrence of microcercous cercariae from *Semisulcospira* sp. in Iwate Prefecture, Japan. According to personal communication from Dr. Saito, the present cercaria seems to be different from the cercaria of Nanophyetidae in the arrangement of penetration gland cells, the size of stylet, and the shape of tail.

Among of the present larvae found in *Bythinella* snails the cercariae are characterized by the internal morphology such as the shape of excretory vesicle, the arrangement of penetration gland cells, and the reaction for toluidine blue. Especially, the shape of excretory vesicle of the present cercaria suggests of the morphological characteristics of the family Nanophyetidae.

The identification of the present larvae will be given after the completion of its life-cycle, though the authors consider that the present cercariae found in *Bythinella* snails and the cercaria of the family Nanophyetidae will be looked as the same morphologically within the limits of our investigation.

## Summary

Trematode larvae (the first and the second generation rediae and the cercaria) being falsely similar to *Paragonimus* found in *Bythinella* snails from Yamaguchi and Ehime Prfectures are demonstrated. The present larvae are very similar to those of *P. miyazakii* in their external appearance, but there are some difference between the two types

of larvae.

They differ each other in the intestine length of the first generation redia, in the pharynx size of the second generation redia, and in the shape of the excretory vesicle of the cercaria. The morphological structures of the present cercaria resemble well those of the family Nanophyetidae. However, the definite identification will remain until the completion of its life-cycle.

**Acknowledgement**

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# ホラアナミジンニナに見られる肺吸虫類似幼虫の特徴について

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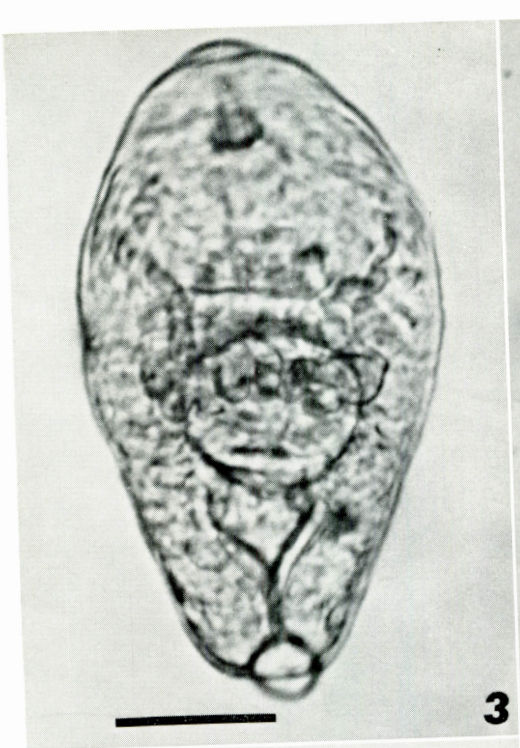
著者らは，さきに宮崎肺吸虫の第1中間宿主としてホラアナミジンニナを報告した（初鹿ら，1966）が，この貝には宮崎肺吸虫のそれと区別すべきレジア，セルカリアが寄生していることを知った．この幼虫は，一見宮崎肺吸虫のものに類似しているが，それよりも全体的にやや小型であるほか，次のような点で区別できる．

すなわち，第1代レジアでは腸管の長さが宮崎肺吸虫のそれよりも短く，第2代レジアでは咽頭の大きさが縦径値＜横径値（宮崎肺吸虫では大部分のものが縦径値＞横径値）の傾向が見られ，セルカリアでは排泄嚢がY字形を呈しているほか，toluidine blue に対する反応にも差異が見られる．

この幼虫は，現在までに山口県で宮崎肺吸虫模式産地と美禰市，愛媛県では宇摩郡西森の貝に見いだされているが，同一個体の貝に両種の混合寄生は認めていない．

この幼虫の種類については未決定であるが，セルカリアにおける排泄嚢の形態的特徴は Nanophyetidae (Wallace, 1935) Dollfus, 1939 のそれに類似している．

今後，この幼虫の発育史を究明するとともに種類を検討したいが，ホラアナミジンニナ寄生の肺吸虫幼虫を検索する際には，この幼虫との区別に注意する必要があると考え，その形態的特徴を記述した．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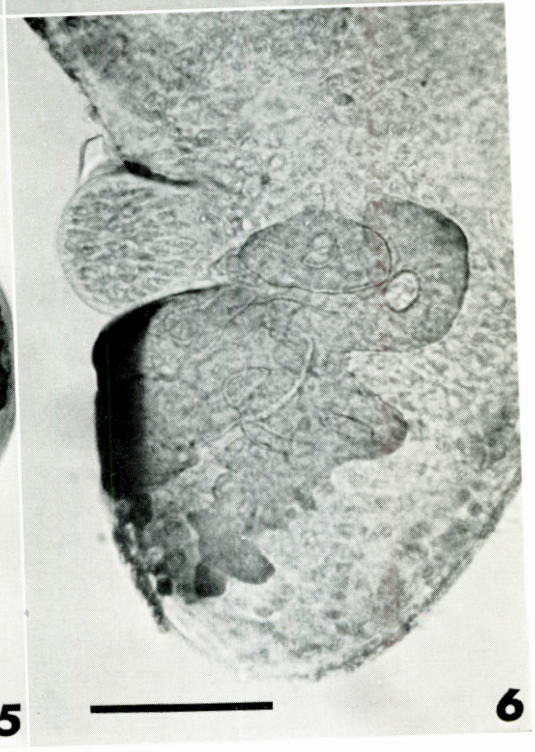
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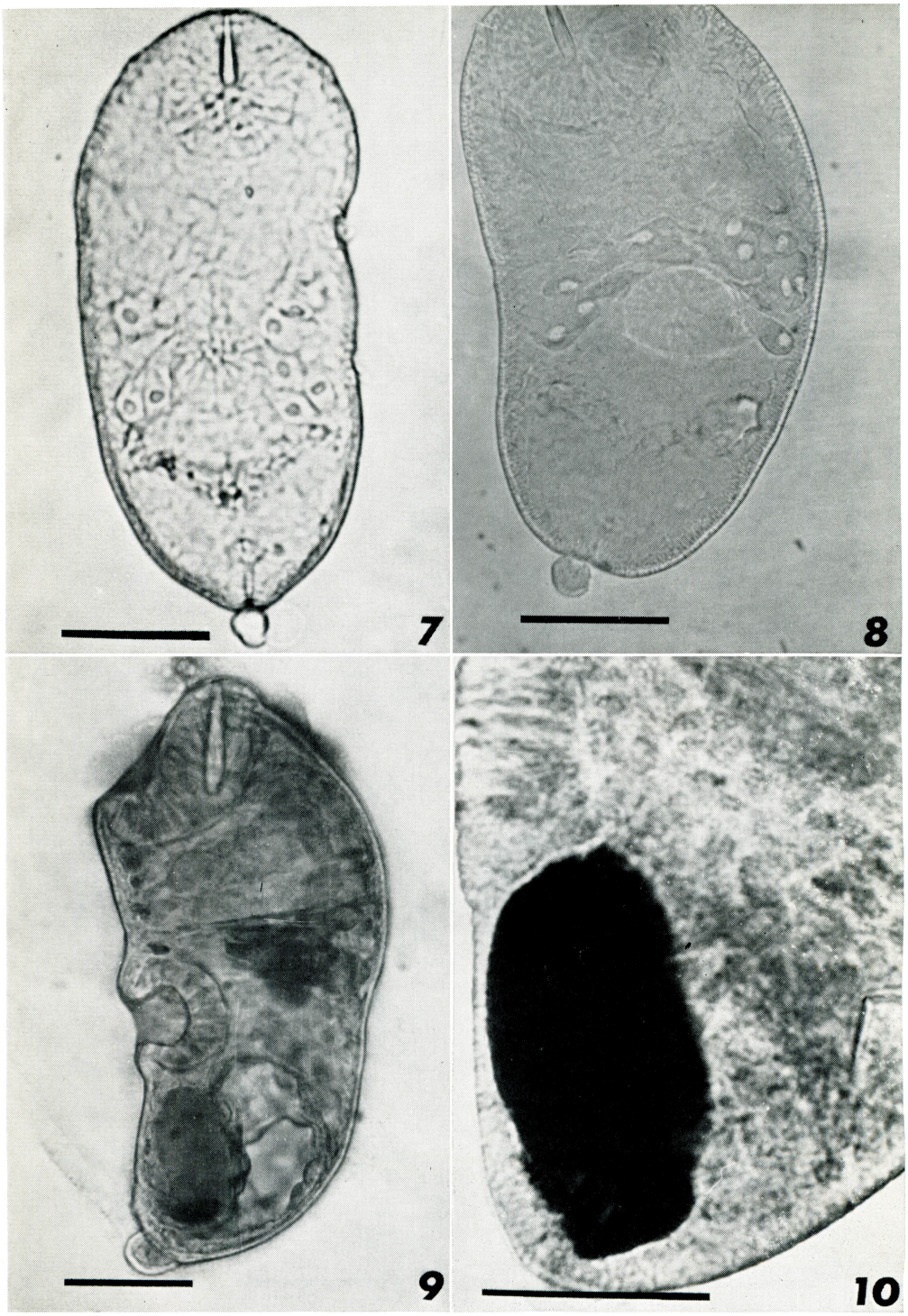
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**Explanation of plates**

Figs. 3–6. Cercariae of *Paragonimus miyazakii* from *Bythinella* snails. (Scale=0.05 mm)

Figs. 3–4 Whole body of cercaria, living specimen. (ventral view)

Fig. 5 Cercaria stained with 1% neutral red, showing the shape of excretory vesicle. (side view)

Fig. 6 Posterior part of cercaria stained with 0.5% toluidine blue. (side view)

Figs. 7–10. Unidentified cercariae, from *Bythinella* snails. (Scale=0.05 mm)

Figs. 7–8 Whole body of cercaria, living specimen. Y-shaped excretory vesicle is the chief note. (ventral view)

Fig. 9 Cercaria stained with 1% neutral red, showing the shape of excretory vesicle. (side view)

Fig. 10 Posterior part of cercaria stained with 0.5% toluidine blue. (side view)